TWO STRUCTURALLY DIFFERENT FAMILIES OF DNA BASE EXCISION REPAIR (BER) PROTEINS DIFFUSE ALONG DNA TO FIND INTRAHELICAL LESIONS

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Base excision repair (BER) proteins, endonuclease III (Nth) and VIII (Nei) from E. *coli* represent two distinct glycosylase families, which recognize and remove damaged DNA bases. One mechanism by which these glycosylases scan for DNA lesions is through a simple, one-dimensional diffusive search. To characterize this search mechanism, we have developed a single molecule assay in near TIRF to image Qdot-labeled, His-tagged Nth and Nei proteins interacting with YOYO-1 stained λ-DNA molecules elongated by hydrodynamic flow between 5μm silica beads. With an *in vitro* glycosylase activity assay, we confirmed that neither YOYO-1 stained DNA nor Qdot labeling significantly affects glycosylase activity. By imaging individual DNA "tightropes", we observed Qdot-labeled glycosylases interacting with DNA by either binding to or diffusing on DNA. With increasing ionic strength (50-500mM Kglutamate), although fewer glycosylases interacted per unit length of DNA, a greater fraction diffused along the DNA. At physiological ionic strength, (150mM KGlu) both Nth and Nei scan DNA for as much as 10 sec with a diffusion constant of ~1.5 x 10^5 bp^2 sec^{-1}, approaching the theoretical limit of rotational diffusion about the DNA helix. At these rates, the activation barrier for rotational diffusion of 0.7 K_bT is slightly below the maximum of ~2 K_bT for efficient target location. We observe no significant difference between Nth and Nei in the rate or mode of their DNA lesion search mechanism. Interestingly, at elevated ionic strengths, both families of glycosylases scan above the theoretical limit for free rotational diffusion (>5 x 10^5 bp^2 sec^{-1}). Therefore, the DNA/glycosylase interface may be optimized for physiological ionic strength, above which the glycosylase search mechanism shifts from rotational diffusion to a one-dimensional diffusion without rotation.